METAMORPHOSIS OF *STICHOPUS CALIFORNICUS* (ECHINODERMATA: HOLOTHUROIDEA) AND ITS PHYLOGENETIC IMPLICATIONS

SCOTT SMILEY

Friday Harbor Laboratories, 620 University Road, Friday Harbor, Washington 98250, and Zoology Department, University of Washington, Seattle, Washington 98195

ABSTRACT

Descriptions of holothurian metamorphosis are based on data from the relatively specialized order Apoda. Metamorphosis for a relatively unspecialized aspidochirote, *Stichopus californicus,* is described here. Metamorphosis in *Stichopus* is characterized by the following features: the madreporic vesicle is a calcite secreting syncytium, not a coelom. *Stichopus* has no separate axocoel and no transient axial complex forms during metamorphosis. The buccal podia form from the water vascular ring, not the radii. The axes of bilateral larval symmetry and the pentaradial adult symmetry are congruent; therefore, the secondarily derived symmetry in holothurians is the pentaradial symmetry of the adult, as in the other extant echinoderms. No axial or visceral torsion occurs during metamorphosis. The enclosed ambulacra form in a manner distinct from that of the ophiuroids and echinoids. Perivisceral coelomic pores develop near the end of metamorphosis, and before the definitive anus forms. Assignment of holothurians with echinoids to the subphylum Echinozoa is discussed.

INTRODUCTION

Detailed comparison of metamorphosis among the extant echinoderm classes led Bury (1895) to propose phylogenetic relationships between them. However, Bury did not propose a phylogenetic position for the holothurians because information on holothurian metamorphosis was incomplete. Today, the phylogenetic relationships between extant echinoderm classes are still disputed. Hyman (1955) and others (Bather, 1900; Beklemischev, 1969), consider holothurians anatomically simple compared to echinoids, because structures such as pedicellariae or movable spines, an axial complex, and Tiedemann's bodies are lacking, and the histological organization of the gonad is relatively uncomplicated. Comparative anatomists interpret this anatomical simplicity of holothurians as primitive and do not group them with echinoids. Paleontologists, however, place holothurians and echinoids in the subphylum Echinozoa (Fell, 1963; Smith, 1984a; Paul and Smith, 1984). Three major arguments support this assignment: (1) both share a globose shape, supposedly the result of similarities in growth patterns. (2) Both have closed ambulacra, and (3), both share calcification around the esophagus in the echinoid lantern and the holothurian aquapharyngeal bulb (Hyman, 1955; Fell, 1963; Smith, 1984a). Embryologists generally agree with paleontologists that holothurian simplicity is derived, and accept the taxon Echinozoa (MacBride, 1914).

Hyman (1955) and Strathmann (1976) show the importance of comparing em-

bryological processes only among indirect developers. This is not because these larvae are necessarily more primitive, but because of the difficulty in making detailed anatomical comparisons between different types of larvae, and because direct developers do not undergo catastrophic morphogenic rearrangement at metamorphosis. Metamorphosis is of particular interest in echinoderm phylogeny because of the questions raised by Bury's (1895) analyses. However, analyses of holothurian embryology and metamorphosis have been hindered by the inability to fertilize ova *in vitro* (Horstadius, 1925; Mortensen, 1931). The two monographs that do provide analyses of metamorphosis of the holothurian auricularia both focus on *Labidoplax digitata* (Semon, 1888; Bury, 1895), which had to be collected from the sea causing critical stages to be missed (Bury, 1895). Bury noted the limited applicability of his study of metamorphosis in *Labidoplax digitata,* an apodan lacking a number of features common to most holothurians (Ekman, 1925; Hyman, 1955). Bury suggested that an analysis of metamorphosis of an auricularia from a different family would help determine the phylogenetic relationships of holothurians.

Several unresolved questions concerning holothurian metamorphosis remain, including: (1) does an axocoelic madreporic vesicle develop from the hydrocoel (Bury, 1895)? (2) Is an axial complex formed during metamorphosis (Cuenot, 1948; Erber, 1983a, b)? (3) Are the buccal podia formed from the radii or the circumesophageal water ring (MacBride, 1914)? (4) Is the larval axis of bilateral symmetry distinct from the adult axis of pentaradial symmetry (Beklemischev, 1969)? (5) Does an incomplete torsion of the viscera occur during metamorphosis (Bury, 1895)? (6) Are the ambulacra covered by a neurulation like overfolding as seen in echinoids (Runnstrom and Runnstrom, 1918; Runnstrom, 1927)?

My investigation into purification of the oocyte maturation hormone of *Stichopus californicus* (Smiley and Cloney, 1985; Smiley, 1986), allowed me to induce meiosis in oocytes and to fertilize these ova. Over the past five years I raised numerous larvae through metamorphosis and obtained all the stages necessary to complete an analysis of metamorphosis. Here, I describe the structure of the auricularia and mid-metamorphic larvae of *Stichopus californicus,* analyze the process of metamorphosis, and answer the questions raised above. Finally, I present arguments that contradict the hypothesis that holothurians and echinoids share a unique ancestor; arguments which are consistent with the hypothesis that holothurian anatomical simplicity is primitive rather than derived.

MATERIALS AND METHODS

Stichopus californicus adults collected by dredging or diving were maintained in aquaria at the Friday Harbor Laboratories of the University of Washington from 1980 through 1985. Ripe ovaries and testes were obtained from late May through early August. Fecund ovarian tubules were ligated, dissected free of the animal, and maintained in freshly filtered seawater at ambient seawater table temperatures for up to a day. Ripe testes were kept covered at 4°C. until used.

An extract of the radial nerves of *Stichopus californicus,* used to induce meiosis (Smiley, 1984; Maruyama, 1985), was prepared as follows. Adults were eviscerated and the muscle layers dissected free from the body wall. The tissue 2 mm on either side of the midline of each longitudinal muscle contained the radial nerve and was excised. This tissue was pooled, heated to 90°C. for 20 minutes, and homogenized in a blender. The mixture was acidified with acetic acid to a final concentration of 0.1 M, then rehomogenized and centrifuged at $18,800 \times g$ for 20 minutes. The supernatant was pooled, frozen, and lyophilized. Ten mg/ml of the dry lyophilizate was added

to culture vessels which contained freshly dissected fecund ovarian tubules. Ovulation and meiosis occurred within four hours after treatment (Smiley, 1984, 1986).

A sperm suspension was made by pipetting 2 mm of testis, in a pasteur pipet, into 10 ml of freshly filtered seawater. A few drops of this suspension was used to fertilize 100 ml of oocytes immediately after germinal vesicle breakdown. Adding sperm to ova after first polar body formation resulted in elevated levels of polyspermy. Immotile sperm were activated by adding aqueous NH_4Cl to a final concentration of 7–10 mM.

Embryogenesis in *Stichopus californicus* follows the pattern described for *Stichopus tremulus* (Holland, 1981). The larvae begin gastrulation about two days after fertilization depending on temperature. About 12 to 18 hours after gastrulation, the mouth has formed and the larvae begin to feed. The larvae were fed with a mixture of the unicellular algae *Dunaliella tertiolecta, Pavlova lutheri,* and *Isochrysis galbana,* grown in 'Algo-Grow' medium (Carolina Biological Supply Co.). Larvae fed a monoculture of any of these algae did not develop well and few completed metamorphosis. Larvae were fed each morning, and the culture water changed each evening with freshly filtered seawater. Growth to competence for metamorphosis took from 18 days to 5 weeks depending on the seawater temperature, the feeding regime, and the source of the eggs.

Larvae were processed for light and transmission electron microscopy by methods previously described (Smiley and Cloney, 1985). Specimens for scanning electron microscopy were fixed in 2% osmium tetroxide in filtered seawater, dehydrated with ethanol and dimethoxypropane. They were dried at the critical point with carbon dioxide, coated with gold, and examined on a JEOL scanning electron microscope. Whole mounts of larvae were made according to Cavey and Cloney (1973).

RESULTS

Metamorphosis of *Stichopus californicus* is quite similar to that of *Labidoplax digitata* (Semon, 1888; Bury, 1895). I define metamorphosis as the transformation of the late larva to the juvenile. Metamorphosis ends when the presumptive adult tissues are in their definitive position. The transformation of the holothurian body during metamorphosis involves morphogenic, histogenic, and organogenic changes, similar to those described for ascidians (Cloney, 1978). Table I summarizes the events of *Stichopus californicus* metamorphosis in temporal order. Because I discuss its phylogenetic implications, I do not describe *Stichopus* metamorphosis in strict temporal order.

Structure of the Stichopus californicus *late auricularia*

The *Stichopus* late auricularia is about a millimeter long and a third of a millimeter wide, with considerable variability between individuals. The body has a complex shape consisting of an oral hood that covers the anterior mid-ventral oral cavity and an anal hood that covers the posterior mid-ventral stomach (Fig. 1). Seen from the side, the oral and anal hoods extend outward from the ventral surface (Fig. 2). The dorsal surface is smooth and has a low convex curvature. Along the margins of the body are small extensions referred to as 'arms'. A continuous strand of ciliated epidermal cells, the ciliated band, lies on the most oblique margin of the body, extending out along the arms. This is the locomotory and feeding organ. The sagittal plane divides the larva into bilaterally symmetric halves, and the frontal plane divides dorsal and ventral surfaces. I term the intersection of these planes the larval axis.

TABLE I

Eve... ... the metamorphosis of Stichopus californicus

Morphogenic metamorphosis 0–4 hours

1. Rapid growth of hydrocoel, formation of anlage of buccal podia, radial vessels, and Polian vesicle.
2. Formation of madreporic vesicle, onset of secretion of madreporic crystal.
3. Change in shape of larva.
4. Reduction in size of larva.
5. Breakup of ciliated band, formation of transverse ciliated rings.
6. Rapid growth of hydrocoel around dorsal side of esophagus.
7. Transient leftwards movement of the opening of oral cavity.
8. Initiation of histolysis of the rectum.
9. Enlargement of coelomic lining of buccal podia.
10. Fusion of left and right ends of hydrocoel at larval mid-ventral line.
11. Protrusion of buccal podia into the oral cavity.

Histogenic metamorphosis 4–24 hours

12. Continued reduction in size.
13. Growth of radial vessels and Polian vesicle.
14. Formation of hyponeural coelom from left and right anterior ends of the somatocoels.
15. Rapid growth of circumoral and radial nervous tissue.
16. Cavitation of tissues superficial to radial nerves to form the epineural sinuses.
17. Movement of opening of oral cavity to anterior end of body.
18. Growth and opening of perivisceral coelomic pore.
19. Migration of madreporic vesicle inward.
20. Fusion of left and right somatocoels.
21. Settlement.

Post-settlement histogenic metamorphosis greater than 24 hours

22. Formation of definitive anus.
23. Histolysis of hydropore and distal hydroporic canal.
24. Initiation of spiculogenesis.
25. Formation of somatic podia.

The larval oral cavity leads posteriorly to the mouth and a muscular esophagus connected to the large spherical stomach by a sphincter valve. The intestine and rectum run anteriorly within the anal hood to the anus from the posterior end of the stomach (Fig. 1). The larva has a left and right somatocoel adjacent to the stomach. The hydrocoel is located on the left side of the larva anterior to the left somatocoel and adjacent to the esophageal stomach juncture (Fig. 2). In the early auricularia the hydrocoel is connected to the left somatocoel, but this connection is broken before the onset of metamorphosis. The hydroporic canal runs from the hydrocoel to the hydropore. The hydropore is located just to the left of the mid-dorsal line at about the level of the esophageal stomach juncture. No adult rudiment occurs in this (Fig. 1, 2), or in any other holothurian auricularia that has been studied (Selenka, 1876; Bury, 1895; Mortensen, 1931). A single calcareous 'body' ossicle is found in the lower left arm. This crystalline structure is surrounded by a thin cellular layer.

Metamorphosis: general aspects

Metamorphosis will occur within 24 hours when evaginations appear along the surface of the hydrocoel (Fig. 10). Metamorphosis can be divided into two phases. The *morphogenic* phase is relatively rapid, it is completed in about 4 hours, and re-
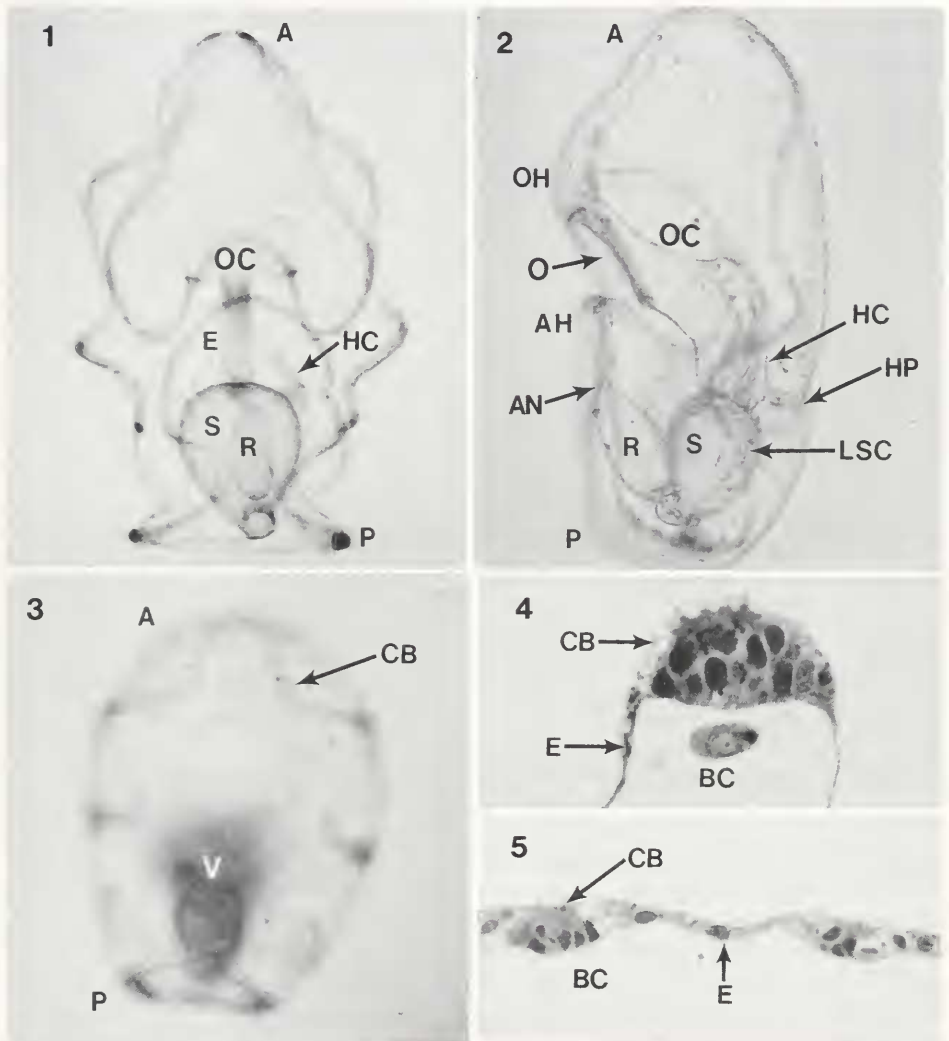
FIGURE 1.   A late auricularia of *Stichopus californicus*. A = anterior, E = esophagus, HC = hydrocoel, OC = oral cavity, P = posterior, R = intestine and rectum, S = stomach. Mag. 225×.

FIGURE 2.   Left lateral view of an earlier auricularia. A = anterior, AH = anal hood, AN = anus, HC = hydrocoel, HP = hydropore, LSC = left somatocoel, OC = oral cavity, OH = oral hood, O = opening of oral cavity, P = posterior, R = intestine and rectum, S = stomach. Mag. 220×.

FIGURE 3.   A mid-metamorphic *Stichopus californicus* larva. A = anterior, CB = ciliated band, P = posterior, V = viscera. Mag. 220×.

FIGURE 4.   Section through the ciliated band and epidermis of a late auricularia. BC = blastocoel, CB = ciliated band, E = epidermis. Mag. 970×.

FIGURE 5.   Section through ciliated bands and the epidermis of a mid-metamorphic larva. BC = blastocoel, CB = ciliated band, E = epidermis. Mag. 700×.

sults in the formation of the *mid-metamorphic* larva (Fig. 3). Most of the morphogenesis is complete when the larva has assumed a spherical shape at its anterior end. This change in shape is correlated with a noticable thickening of the epidermis (Figs. 4, 5).

The second phase of metamorphosis, the *histogenic* and *organogenic* phase is slower, requiring about 24 hours to complete, and transforms the mid-metamorphic larva to the *pelagic juvenile* (Fig. 14). I have avoided using the term doliolaria for this stage because it is equally applied to the directly developing larvae of holothurians and crinoids as well as the post-metamorphic stage of indirectly developing holothurians. The entire process of metamorphosis is continuous. The stages described here are transitory, grade into one another, and are primarily used for convenience.

Metamorphic transitions during the morphogenic phase

Changes in shape and size. The most obvious difference between the auricularia and the mid-metamorphic larva is the loss of the complex shape (Figs. 3, 7). This change begins at the posterior end of the larva and progresses anteriorly. It is a rapid change, often taking less than an hour to accomplish, and it results in the nearly spherical early mid-metamorphic larva. The transition includes the release of the deformation of the epidermis that contributed to the complex shape of the auricularia (Figs. 1, 3). Associated with this, the uniformly distributed fibers in the blastocoelic connective tissue of the auricularia (Fig. 6) are noticably more dense than in the mid-metamorphic larva (Fig. 7). The late mid-metamorphic larva is approximately two-thirds the length and half the width of the auricularia. The change in size of the auricularia also begins at the posterior end of the larva and gradually makes its way forward. This shrinkage in size is continuous throughout metamorphosis.

Breakup of the ciliated band. Concomitant with the changes in shape and size of the larva is the rearrangement of the ciliated band (Figs. 1, 3). The ciliated band forms incomplete transverse rings around the larva during morphogenic metamorphosis. There are breaks in the rings at the mid-ventral and mid-dorsal lines (Fig. 14). The formation of these rings begins at the posterior end and progresses anteriorly until at the completion of metamorphosis there is a total of five rings. The fourth ring maintains a distinct looped shape until late in metamorphosis when the mid-ventral loop finally separates off and becomes the fifth, most anterior ring (Fig. 3).

Torsion, and the axes of larva and adult. The larval axis, the line of intersection between the sagittal and dorso-ventral planes of the auricularia larva, is identical to the anterior-posterior axis of the mid-metamorphic larva, the pelagic juvenile, and with the anterior-posterior or oral-anal axis of the adult. The larval and adult axial relationships, the pattern of formation of the enterocoel, and the definitive configuration of the larval coeloms for four of the extant echinoderm classes is depicted in Figure 16. Because no extant crinoid has an indirectly developing larva, this class is excluded. Since the axis of symmetry of the larva and juvenile are the same in *Stichopus,* no axial torsion (the twisting of the animal's functional axis) occurs in metamorphosis. Neither does *Stichopus* undergo a visceral torsion (the condition particularly pronounced in ophiuroids, where the viscera twist during metamorphosis so that the line of fusion of the somatocoels is perpendicular to the oral-aboral adult axis) (Mac-Bride, 1914; Hyman, 1955).

The oral cavity. The opening to the oral cavity of the *Stichopus* larva becomes slightly displaced to the left with respect to the larval axis during the first phase of metamorphosis. This change is transitory; the position of the opening relative to the axis is restored as the larva continues to shrink. The shape of the oral cavity also changes during metamorphosis, due to differential changes in the topology of the larval epidermis. Previously, the oral cavity was called the "vestibule," and the opening of the oral cavity, the "mouth." These terms imply homology with the developing rudiment of echinoids and are not used here. The true mouth of both the auricularia

and the mid-metamorphic larva is situated at the bottom of the oral cavity, at the anterior end of the larval esophagus (Fig. 1). The mouth and the oral cavity are present in the auricularia and do not form by invagination as Bury (1895) suggested. The appearance of invagination is produced by changes in the epidermis that occur during metamorphosis. No rudiment develops near the oral cavity, or anywhere else, in this holothurian. When metamorphosis is complete, the oral cavity becomes the oral sheath surrounding the buccal podia of the juvenile (Fig. 15).

Changes in the larval gut. During the morphogenic phase in *Stichopus californicus,* the larval intestine and rectum (Fig. 1) lose their tubular form and begin to histolyze (Figs. 18, 19). The larval anus initially remains open during morphogenic metamorphosis (Fig. 8), but it soon closes. The definitive intestine begins to form at the posterior end of the stomach during the histogenic phase of metamorphosis, but the definitive anus is not fully formed before settlement. The larval stomach changes considerably during the morphogenic phase (Figs. 6, 7). The stomach wall becomes thicker and the entire structure shrinks to a fraction of its former size. No marked histolysis occurs other than in the tissues of the intestine, rectum, and anus.

Development of the hydrocoel and water vascular system. Just prior to the onset of metamorphosis five distinct lobes or evaginations develop on the anterior surface of the hydrocoel (Fig. 10). The lobes greatly enlarge during the first stage of metamorphosis (Figs. 8, 9), but remain on the anterior surface of the hydrocoel. The evaginations can be followed during metamorphosis (Figs. 9, 17), as they become the coelomic lining of the primary buccal podia. Once they have grown to just beneath the epidermis of the oral cavity, they appear to induce the epidermis of the oral cavity to grow out around them (Figs. 9, 17). Prior to settlement, the buccal podia can protrude from the oral cavity (Fig. 15). The juvenile's buccal podia attach to the substratum during settlement in *Stichopus.* At no time during the ontogeny of the buccal podia is their hydrocoelic lining directly connected to an ambulacral water vascular canal.

The hydrocoel itself also enlarges rapidly during this morphogenic stage, principally by division of the coelomic epithelial cells. It grows around the esophagus (Fig. 9) from the left to the right side of the larva, to meet itself near the larval mid-ventral line. When its left and right ends have fused, the hydrocoel becomes the circumesophageal water vascular ring. The five evaginations that will become the ambulacral water vascular radial canals develop on the posterior surface of the hydrocoel, between the evaginations of the buccal podia. The mid-ventral radial water vascular canal of *Stichopus* grows considerably faster than the others (Fig. 18). This growth primarily involves the posterior elongation of the ambulacral water vascular canals, which are superjacent to the developing perivisceral coelomic peritonea and subjacent to the connective tissue compartment of the body wall. At no time are the developing radial canals of the water vascular system close to the epidermis of the larva. An eleventh evagination of the hydrocoel forms the Polian vesicle of the pelagic juvenile.

The madreporic vesicle. The madreporic vesicle (Fig. 13) is spherical, forms around the hydroporic canal in the late auricularia larva, and is an example of a histogenic change associated with the first phase of metamorphosis. The madreporic vesicle surrounds the developing madreporic crystal (Figs. 10, 11), which has the shape of a fine filigree and is composed of calcareous material (Fig. 11), judged by polarizing microscopy. This vesicle is first visible just beneath the larval epidermis, separated from the larval hydrocoel by the length of the hydroporic canal. By the end of the morphogenic phase, the vesicle has withdrawn into the interior of the animal. This inward movement is greater than the diminishment in larval size; consequently, the motion cannot be explained by the reduction in larval size alone. The most proximal portion of the larval hydroporic canal becomes the adult stone canal in *Stichopus*
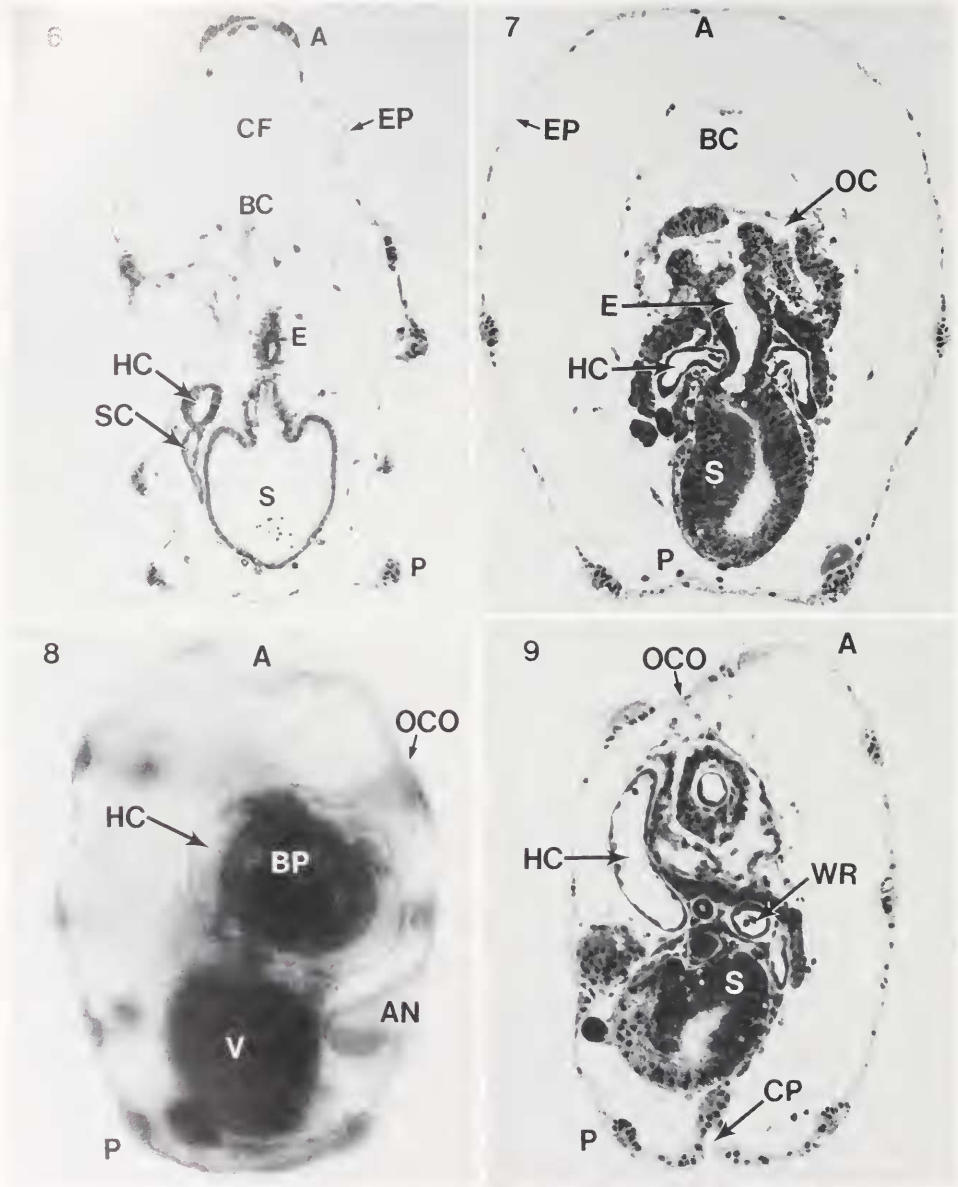
FIGURE 6.   Frontal section through a late auricularia. A = anterior, BC = blastocoel, CF = connective tissue fibers, E = esophagus, EP = epidermis, HC = hydrocoel, P = posterior, S = stomach, SC = somatocoel. Mag. 160×.

FIGURE 7.   Frontal section through a mid-metamorphic larva. A = anterior, BC = blastocoel, E = esophagus, EP = epidermis, HC = hydrocoel, OC = oral cavity, P = posterior, S = stomach. Mag. 220×.

FIGURE 8.   Whole mount of a metamorphosing *Stichopus* larva. A = anterior, AN = closing anus, BP = forming buccal podia, HC = hydrocoelic lining of buccal podia, OCO = opening of oral cavity, P = posterior, V = viscera. Mag. 330×.

FIGURE 9.   Saggital section through a late metamorphosing larva. A = anterior, CP = perivisceral coelomic pore, HC = hydrocoel, OCO = opening of oral cavity, P = posterior, S = stomach, WR = water vascular ring canal. Mag. 250×.

californicus. Cilia within the hydropore stop beating prior to settlement and the hydropore closes soon after the larva settles.

Previous investigators held that the madreporic vesicle was the holothurian axocoel (see Bury, 1895). In *Stichopus californicus,* only a thin cellular layer surrounds each of the fine calcareous filaments of the madreporic crystal (Fig. 13). Transmission electron microscopy reveals that this layer is part of a single cell and not an epithelium (Fig. 20). The perikarya of the cells forming the madreporic vesicle syncytium lie in its center and are morphologically similar to the perikarya of the syncytium which secretes the larval skeleton in echinoids (Gibbins *et al.,* 1969). I found no evidence of intercellular junctions between the syncytium of the madreporic vesicle and the epithelial cells of the hydroporic canal, a condition consistent with the observation that the madreporic vesicle migrates along the hydroporic canal during metamorphosis. The outer surface of the madreporic vesicle is associated with a prominent external lamina, similar to the external lamina that surrounds ossicle secreting syncytia in adults of the holothurian *Leptosynapta clarki* (Stricker, 1985). The structure of the madreporic crystal supports the hypothesis that it is secreted within intracellular vacuoles of the syncytium (Figs. 13, 20), reminiscent of the secretion of the larval skeleton of ophiuroids and echinoids (Okazaki and Inoué, 1976).

The pelagic juvenile: histogenic metamorphosis

The changes required to make a pelagic juvenile (Fig. 14) from the mid-metamorphic larva are not substantial. The pelagic juvenile is more cylindrical and about half the size of the mid-metamorphic larva by the time of settlement. Upon completion of metamorphosis, the anlagen of all the juvenile tissues are in their appropriate locations. Consequently, most of the changes during this period involve tissue growth (see Table I). The larva continues to shrink during this second phase of metamorphosis. The net result of this shrinkage is the further condensation of the body wall connective tissue compartment (Figs. 7, 9). Growth of the left and right somatocoels, coupled with decrease in the size of the larva, causes the somatocoels to become closely applied to one another while lining the body cavity and surrounding the gut. When the somatocoelic epithelia fuse together, these cavities become the perivisceral coelom. The mesenteries formed in the fusion of the somatocoels are parallel to the larval axis.

Development of the perivisceral coelomic pore. A pore is first visible in scanning electron micrographs of the posterior surface of the pelagic juvenile (Fig. 12). This is not the anus but a perivisceral coelomic pore (Fig. 9), which is connected to the right somatocoel. The connection to the somatocoel was ascertained only after examination of serial semi-thin cross sections. The single coelomic pore opens before histolysis of the larval rectum is complete, and the definitive anus forms.

The enclosure of the ambulacra. In the mid-metamorphic *Stichopus* larva, the elongating water vascular radial canals grow posteriorly from the circumesophageal water vascular ring canal to deep within the larva. The radial canals are a considerable distance from the epidermis, separated by the connective tissue compartment of the body wall (Figs. 9, 18). Tissues that will contribute to the radial nerves of the juvenile are superjacent to each elongating radial canal and extend from the circumesophageal nerve ring to near the end of the radial canal (Figs. 18, 21, 22). Superjacent to the anlage of the radial nerve and at a more anterior location, is an area where the epineural sinus first appears (Fig. 22). I found no evidence of an epineural sinus beneath the floor of the oral cavity, and superficial to the circumesophageal nerve ring in any specimens, although it occurs in this position in adults (Hyman, 1955). The epineural
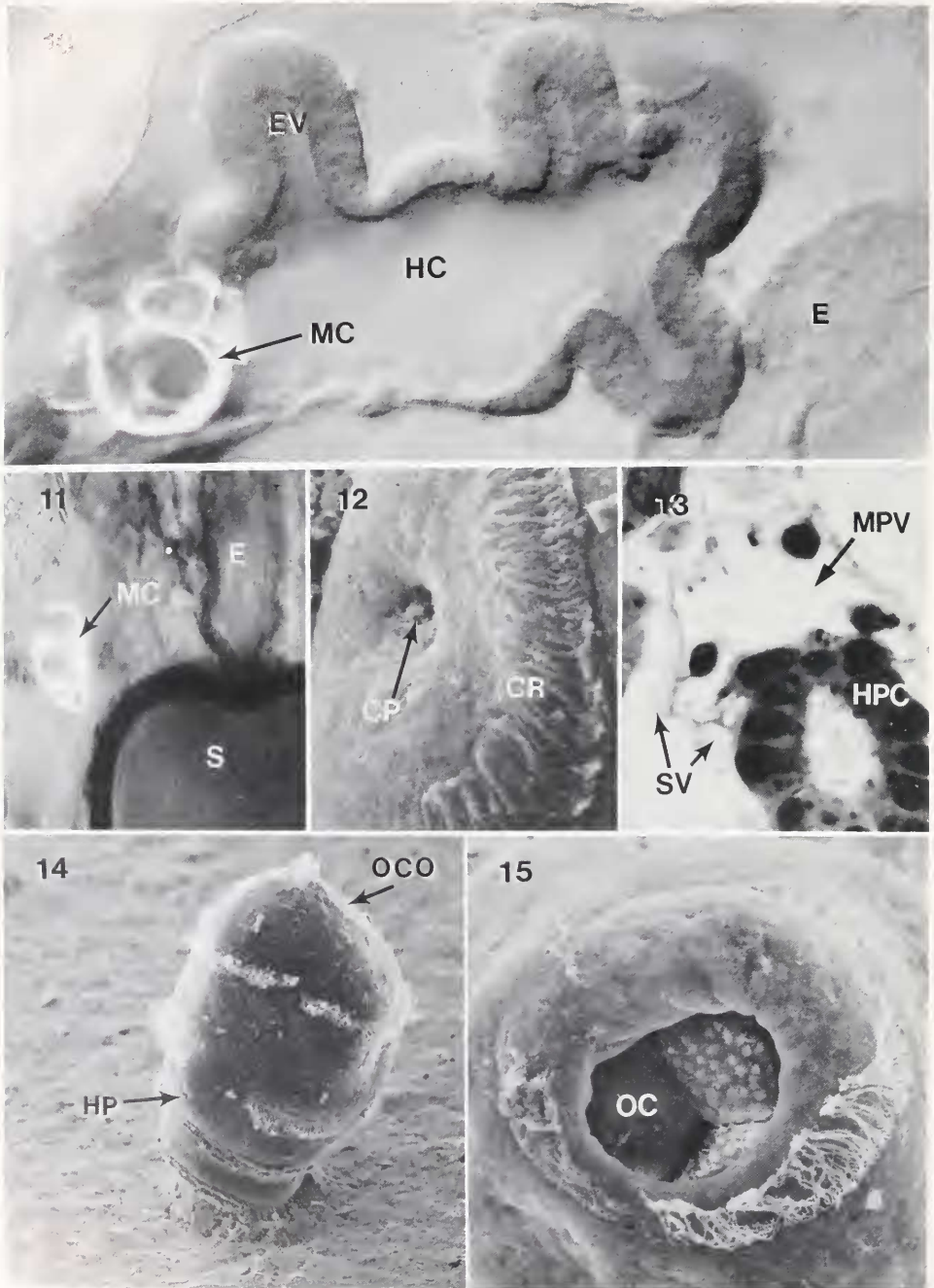
FIGURE 10. Doubly exposed polarizing and differential interference contrast micrograph of the larval hydrocoel and madreporic crystal. E = esophagus, EV = evaginations of the hydrocoel, HC = hydrocoel, MC = madreporic crystal. Mag. 1230×.

FIGURE 11. Doubly exposed polarizing and DIC micrograph of the madreporic crystal surrounding the hydroporic canal. Note that the madreporic crystal has the shape of a fine filigree. E = esophagus, MC = madreporic crystal, S = stomach. Mag. 620×.
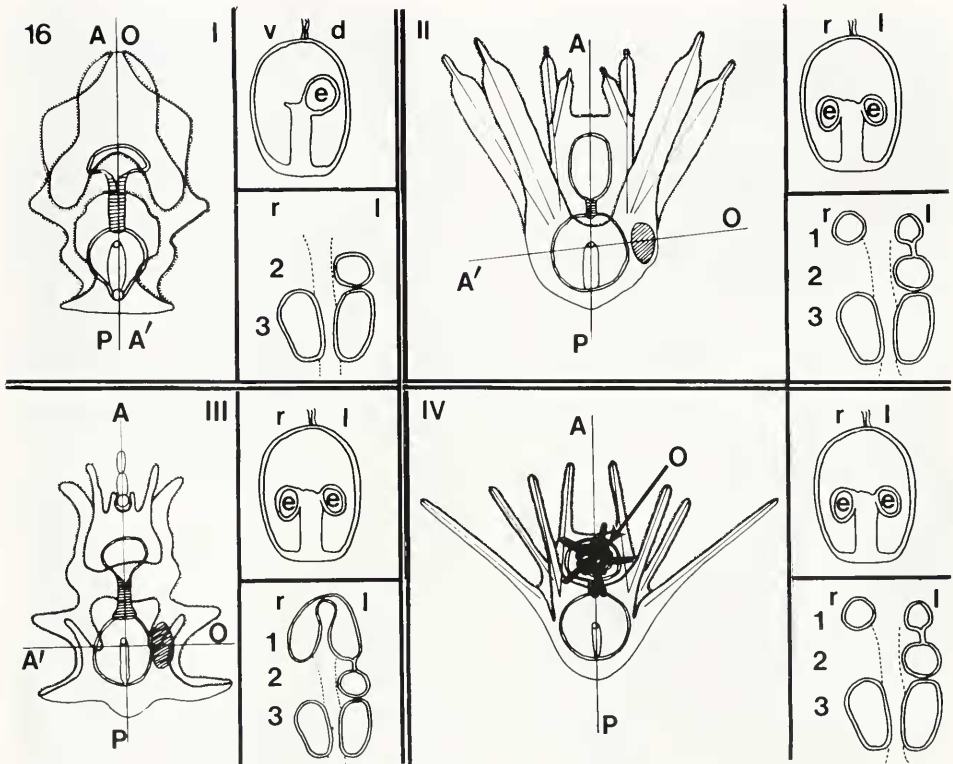
FIGURE 16.   Larval and adult axial relationships as well as coelomic configurations at formation and in the definitive larva in four classes of echinoderms. I = Holothurians, II = Echinoids, III = Asteroids, and IV = Ophiuroids. The axis of pentaradial symmetry of ophiuroids is perpendicular to the plane of the page, but also perpendicular to the larval axis. The position of the adult rudiment is indicated by diagonal lines. A—P indicates the larval axis, O—A′ indicates the axis of the adult. r, l = right, left. d, v = dorsal, ventral. e = enterocoelomic vesicle. 1 = Axocoel. 2 = Hydrocoel. 3 = Somatocoel.

sinus of *Stichopus* appears to be formed by cavitation of tissues superficial to the presumptive radial nerve (Figs. 18, 22). In longitudinal sections through developing ambulacra, the tissue that will form the epineural sinus is visible as a single layer of squamous cells superjacent to the radial nerve anlage. I found no evidence of epineural flaps, nor of their folding over, either along the larval body, or on the floor of the oral cavity. Finally, no indications of the hyponeural sinus were found along the ambulacra, suggesting that this structure develops after metamorphosis.

FIGURE 12.   SEM of the posterior of a late metamorphosing larva. CP = perivisceral coelomic pore, CR = posterior most ciliated ring. Mag. 990×.

FIGURE 13.   Section through the madreporic vesicle. HPC = hydroporic canal, MPV = madreporic vesicle, SV = secreting vacuoles of the syncytium. Mag. 1750×.

FIGURE 14.   SEM of a right rear view of a pelagic juvenile larva of *Stichopus* showing the incomplete transverse ciliated rings. The apparent apical tuft is debris, a preparation artifact. HP = hydropore, OCO = opening of the oral cavity. Mag. 280×.

FIGURE 15.   SEM of the opening of the oral cavity of a pelagic juvenile larva, showing the tips of the buccal podia. OC = oral cavity. Mag. 790×.
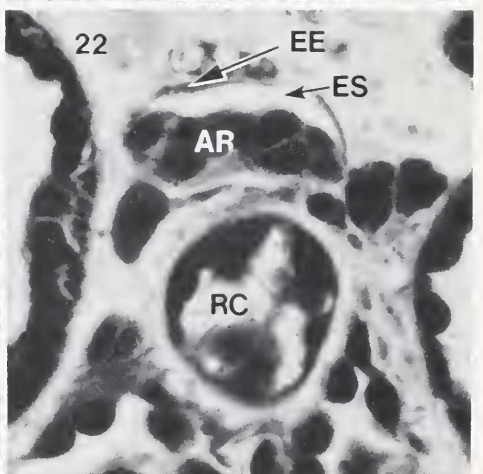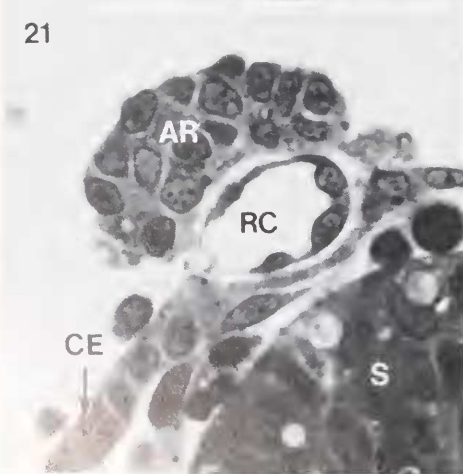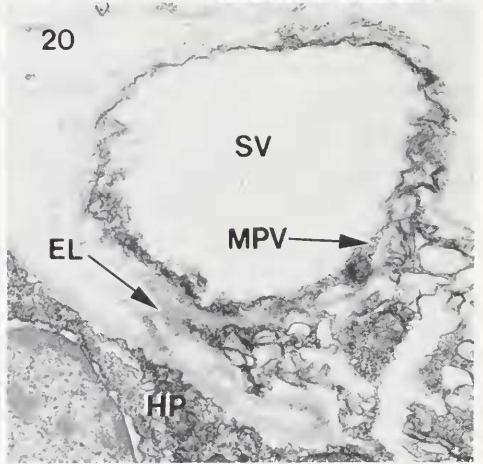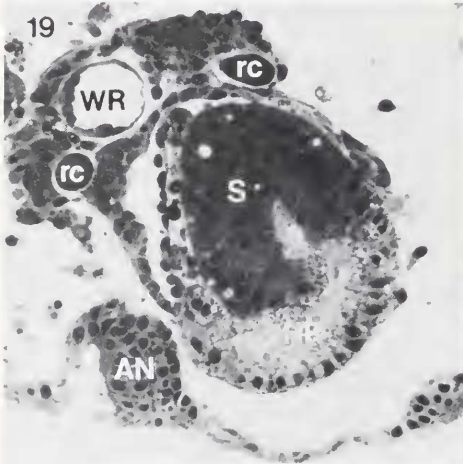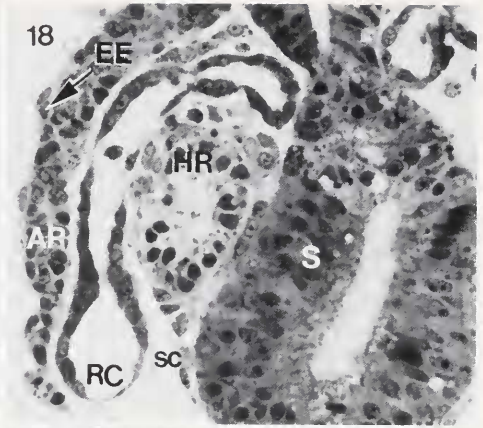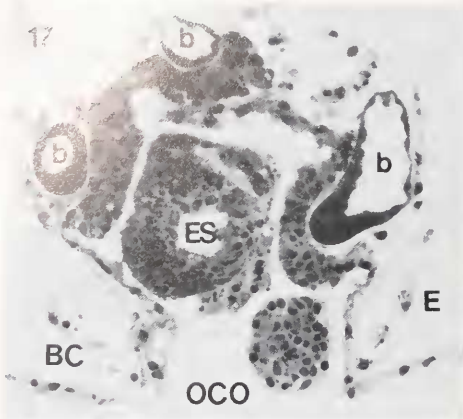
No axial complex or gonad is formed during metamorphosis

No axial complex forms during metamorphosis in *Stichopus californicus*. The transient presence of this organ in holothurians was suggested by Cuenot (1948). Neither is the connective tissue component of the axial complex, the axial gland (Erber, 1983b), present in newly metamorphosing *Stichopus californicus,* suggesting that this structure originates later in development. There is no primordium of the gonad in newly metamorphosed animals, consistent with reports that the onset of echinoderm gonadogenesis occurs some months after settlement (Nieuwkoop and Sutasurya, 1981).

DISCUSSION

Stichopus californicus metamorphosis follows the general pattern described for *Labidoplax digitata* by Semon (1888) and Bury (1895). This metamorphosis differs from previous accounts in that: (1) the madreporic vesicle, a structure presumed to be an axocoel by Bury (1895), is instead a syncytium. The madreporic crystal is secreted within intracellular vacuoles of this syncytium. (2) No transient axial complex forms during metamorphosis. (3) The coelomic linings of the buccal podia are derived from the circumesophageal water vascular ring canal and not from the radial canals. (4) The axes of bilateral and pentaradial symmetry of the juvenile are congruent, and are identical to the larval axis. (5) There is no torsion of the viscera during metamorphosis of this holothurian. (6) There is no folding over of epineural tissue flaps to enclose the ambulacra in *Stichopus;* the epineural sinus is formed by cavitation of tissue layers superficial to the radial nerves. (7) A perivisceral coelomic pore, which connects the right somatocoel to the sea, forms before the definitive anus, during the time the lower larval gut is being resorbed.

Mechanisms of morphogenic metamorphosis

My analysis of metamorphosis in *Stichopus californicus* suggests two hypotheses concerning the mechanisms controlling the process. First, a decrease in the stiffness of the blastocoelic connective tissue occurs at the onset of the first phase of metamorphosis. This decrease allows the shape of the larva to change from the complex form of the auricularia to the roughly spherical mid-metamorphic larva. Changes in the stiffness of holothurian connective tissue have been documented for a number of species under a variety of conditions (Smith and Greenberg, 1971; Emson and Wilkie, 1980; Motokawa, 1984). While these changes appear to be under nervous control, their biochemical basis is not resolved. If the blastocoelic connective tissue were

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of radial nerve, EE = epineural sinus epithelium, HR = histolyzing intestine and rectum, RC = radial canal, S = stomach, SC = somatocoel. Mag. 650×.

FIGURE 19. Section through histolyzing intestine and rectum of a metamorphosing larva. AN = anus, HR = histolyzing tissue, RC = radial canal, S = stomach, WR = water vascular ring canal. Mag. 440×.

FIGURE 20. TEM through a secreting vacuole of the madreporic vesicle. EL = external lamina, HP = hydroporic canal, MV = madreporic vesicle, SV = secreting vacuole. Mag. 15,330×.

FIGURE 21. Cross section through the posterior region of the elongating mid-ventral radial canal of a metamorphosing larva. AR = anlage of the radial nerve, CE = perivisceral coelomic epithelium, RC = radial canal, S = stomach. Mag. 1570×.

FIGURE 22. Section through a more anterior region of a developing ambulacrum. AR = anlage of the radial nerve, EE = epineural sinus epithelium, ES = epineural sinus, RC = radial canal. Mag. 2100×.

stiff in the auricularia, it would allow the animal to maintain its complex shape with a minimum of energy expended. The complex shape of the auricularia is likely to facilitate feeding and locomotion driven by the ciliated band (Strathmann, 1971). The observation that the blastocoelic connective tissue fibers are more dense in the auricularia than in the mid-metamorphic larva is consistent with this hypothesis.

The second hypothesis is that changes in the shape of epidermal cells contribute to the tension within the epidermis. In this case, epidermal tension works concurrently with the changes in the stiffness of the connective tissue to produce the spherical mid-metamorphic larva. I have documented changes in the shape of the larval epidermal cells, from squamous to low cuboidal, during metamorphosis (Figs. 4, 5). I interpret these changes to be a reflection of the tension within these cells. Continued tension may be responsible for the gradual reduction in size that accompanies the transformation of the mid-metamorphic larva to the pelagic juvenile.

The morphogenic movements which occur in the metamorphoses of asteroids, ophiuroids, and echinoids are exceedingly complex (MacBride, 1914; Hyman, 1955), and cannot be described easily here. Comparison of metamorphic morphogenesis in these animals with that in *Stichopus californicus* shows that there are both fewer movements and less complicated mechanisms required to explain them in holothurians.

Symmetry and the patterns of holothurian metamorphosis

The auricularia larva, like all indirectly developing echinoderm larvae, is distinctly bilateral. The bilateral symmetry probably makes for economy in the control of development, as well as economy in locomotion and feeding (Strathmann, 1971). Like all adult echinoderms, adult holothurians have pentaradial symmetry. The hydrocoel organizes this pentaradial symmetry beginning at metamorphosis, and maintains it during growth through the influence of the radial vessels of the water vascular system.

Holothurians also bear a bilateral symmetry in the adult, a symmetry most evident in the division of the body into a ventral trivium and a dorsal bivium. It is usual, however, to refer to the bilaterality of adult holothurians as a secondary acquisition, underlining the supposedly derived character of the class (Hyman, 1955). A unique difference in holothurian metamorphosis is that there is no axial torsion of the body to a position 90 degrees from the larval axis, as is the case in the asteroids and echinoids (see Fig. 16). Neither is there torsion of the viscera to a position 90 degrees off the larval axis, as is the case in ophiuroids (Bury, 1895). Metamorphic torsion can be observed or inferred in the other classes either from the location of the adult rudiment or from the configuration of the coelomic mesenteries supporting the gut (Bury, 1895; Hyman, 1955).

Bury (1895) interpreted the leftwards motion of the opening of the oral cavity in *Labidoplax* as a slight or vestigial torsion during early metamorphosis, as does Ohshima (1921). I corroborate Bury's observation of this movement in *Stichopus*, but I suggest that the transient leftwards displacement of the opening of the oral cavity is due to the growth of the hydrocoel around the larval esophagus during the first phase of metamorphosis, rather than to an ancestral reminiscence of visceral torsion as proposed by Bury (1895) and elaborated by MacBride (1914). In *S. californicus,* the oral cavity can be followed from the auricularia through to settlement, but Bury (1895) presumed that the oral cavity formed at metamorphosis, and used this observation to support a homology between the echinoids and holothurians. Bury's misinterpretations were probably due to his inability to culture *L. digitata in vitro,* causing

him to miss the intervening stages. Not all echinoids have a vestibule (Emlet, pers. comm.), indicating that echinoid development may not be as distinct from that of asteroids as previously thought (MacBride, 1914).

I have found no accounts of the position of the coelomic mesenteries of any holothurian which could be interpreted to reflect a torsion of the viscera about the body axis in metamorphosis. During *Stichopus* metamorphosis, the bilaterality of the juvenile is congruent with, and derived from, the bilaterality of the auricularia. Therefore, the assertion that the bilaterality of holothurians is secondary is incorrect. The symmetry acquired secondarily in holothurians, as in all echinoderms, is the pentaradial symmetry of the adult.

The shift of the functional axis during metamorphosis of asteroids, ophiuroids, and echinoids entails tremendous changes. These changes are correlated with the presence of an adult rudiment on the larva, which dominates the development of the post-metamorphic juvenile; the greater preponderance of the larval tissues being lost or phagocytized (Chia and Burke, 1978). The ingression of strictly adult tissues into the larval body often indicates that temporal compression in development has occurred (Gould, 1977). No axial transformations occur during holothurian development, either in the metamorphosis of indirectly developing species or in the growth and settlement of direct developers (Semon, 1888; Bury, 1895; Ohshima, 1921; Inaba, 1930). Nor are there any reports of the development of a rudiment on the larvae of indirectly developing holothurians (Selenka, 1876; Semon, 1888; Bury, 1895; Mortensen, 1931; Rustad, 1940). In the metamorphosis of *Stichopus,* only the larval intestine, rectum, and anus are resorbed, and the majority of all holothurian larval tissues are directly incorporated into the juvenile (Semon, 1888; Bury, 1895). The absence of an adult rudiment on the holothurian auricularia larva indicates that there has been no massive temporal compression of development. Therefore, when we compare the patterns of metamorphosis in holothurians with those in asteroids, ophiuroids, and echinoids, we contrast untorted, simple, and continuous morphogenic movements showing no evidence of developmental compression, with torted, complex, and discontinuous morphogenic movements where there is good evidence for temporal compression.

These arguments support the conclusion that the patterns of holothurian metamorphosis are considerably more simple than the patterns described for asteroids, ophiuroids, and echinoids. The available data are not consistent with the hypothesis that the simplicity of holothurian metamorphosis is derived. If this were the case we would expect to find vestiges of a more complex mechanism of morphogenic movements, of axial transformation, or of an adult rudiment in holothurian metamorphosis. The hypothesis that the simplicity of holothurian metamorphosis might be derived and yet leave virtually no trace, does not offer explicit testable predictions. I do not believe that such extraordinary compression of development could occur and be undetectable. Finally, the patterns of metamorphosis in *Stichopus* do not support the hypothesis that holothurians and echinoids are closely related.

The internal madreporite and coelomic pores

In *Stichopus californicus,* the madreporite is internal and is the terminus of the stone canal. This is also the case in most other holothurians including those groups traditionally thought to represent the ancestral holothurian stock, the aspidochirotes and dendrochirotes (Eckman, 1925; Hyman, 1955). There is some controversy as to the definitive location of the madreporite in holothurians (Erber, 1983a) because in the elasipods the madreporite is lodged in the body wall and in some molpadids the

larval hydropore fails to close (Hyman, 1955). Most authorities acknowledge that the condition in these two orders is derived (Ekman, 1925; Hyman, 1955). There is little question, however, that the internal madreporite in holothurians functions to allow passage of water vascular and coelomic fluids (Nichols, 1969). Crinoids, as far as is known, have neither a madreporite nor a madreporic vesicle, but the ends of their many stone canals hang freely in the perivisceral coelom, and it is likely that fluids pass through the open ends of the stone canals (Bather, 1900). The madreporite in asteroids, ophiuroids, and echinoids is external and also functions in the passage of fluid (Hyman, 1955). The inner surface of the madreporite is derived from the right axocoel in these echinoderms and it surrounds the terminus of the stone canal (Hyman, 1955). Only a few extinct echinoderms possessed an external madreporite, including all the ophiocystioids, although many had a hydropore as adults (Bather, 1900; Hyman, 1955; Nichols, 1969). The presence of an external madreporite in the ophiocystioids, asteroids, ophiuroids, and echinoids, and its absence in all other echinoderm classes suggests that its presence is a derived condition.

Prior to the formation of the definitive anus in *Stichopus,* a coelomic pore develops which connects the right somatocoel with the exterior. The early development of this pore suggests a functional significance in the adult. These pores have recently been described for adult *Stichopus californicus* (Shinn, 1985a), and their fine structure noted (Shinn, 1985b). While there is no evidence that the other eleutherozoans have coelomoducts, extant and extinct pelmatozoans do possess pores which may have functioned in conveying seawater to the perivisceral coelom. Crinoids have ciliated pores which are perivisceral coelomoducts (Hyman, 1955), and many extinct echinoderms also had respiratory coelomic pores passing through the body wall (Bather, 1900). This suggests that pores connecting the perivisceral coelom with the sea may be primitive in echinoderms. This conclusion is further strengthened by the observation that coelomic pores are correlated with the absence of an external madreporite. In summary, these arguments contradict the hypothesis that echinoids and holothurians shared a common ancestor, and supports the hypothesis that the presence of an internal madreporite in holothurians is primitive rather than derived.

Closed ambulacra

The ambulacra of holothurians are covered by the body wall, and an epineural sinus is found superficial to the radial nerves (Hyman, 1955). This morphology is similar to that of ophiuroids and echinoids, but distinct from the naked ambulacra of the crinoids and asteroids (Hyman, 1955). The presence of closed ambulacra has been used to argue an evolutionary relationship between the holothurians and the echinoids (Fell, 1963; Smith, 1984a). However, in echinoids and ophiuroids, the ambulacra are closed by the folding over of epineural flaps of tissue in a manner reminiscent of the neurulation of vertebrate embryos (MacBride, 1914). In *Stichopus californicus,* the ambulacral epineural sinus forms by cavitation of the tissue layers superficial to the radial nerves. I found no evidence of a neurulation-like event either at the circumesophageal nerve ring or along the radial nerves. Hyman (1955) claimed that Runnstrom and Runnstrom (1918) and Runnstrom (1927) reported that epineural flap overfolding enclosed the epineural sinus of several holothurians. In fact, these investigators only suggest this process as a likely possibility; they did not observe such an event (Runnstrom and Runnstrom, 1918; Runnstrom, 1927).

I suggest that one reason for the epineural overfolding of ectodermal tissue flaps during the closure of echinoid ambulacra may be to invaginate ectodermally derived tissues to a sub-epithelial location, a process required as a consequence of the radical

metamorphosis in these animals. The ectodermal tissues then could be recruited to form the circumesophageal and radial nerves. Holothurians already have nerve cells in the area of the mouth as reported by Burke *et al.* (1986). Thus, an invagination may be unnecessary in this class. Since the epineural sinus of holothurians is formed by a mechanism different from that found in the echinoids and ophiuroids, closed ambulacra do not necessarily indicate a close evolutionary relationship between these classes and the holothurians (Hyman, 1955; Fell, 1963; Smith, 1984a). Nichols (1967) suggested that closed ambulacra may have arisen more than once in the echinoderms. The evidence from *Stichopus californicus* supports this suggestion. The different modes of enclosure of the ambulacra in holothurians and echinoids directly contradict the hypothesis that holothurians and echinoids share a common ancestor. However, we cannot infer if the condition in holothurians is primitive or derived from the available evidence.

Coelomic organization

The notion of a bilaterally paired, tripartite coelomic organization of larvae has been deeply integrated into phylogenetic studies of echinoderms (Bather, 1900; Hyman, 1955). The homologies of both the water vascular and perivisceral coeloms in the extant classes pose few problems (Bather, 1900; MacBride, 1914; Hyman, 1955). But, it is necessary to explain the relationship between the single anterior coelomic compartment of holothurians and the several anterior coelomic compartments of the asteroids, ophiuroids, and echinoids. In nearly all holothurians the original enterocoelic vesicle (Fig. 16), which gives rise to the anterior coelom, is single and arises dorsally (Selenka, 1876; Ohshima, 1921; Inaba, 1930; Rustad, 1940). In contrast, the enterocoelic vesicles of asteroids, ophiuroids, and echinoids are paired and arise laterally (MacBride, 1914; Hyman, 1955).

The madreporic vesicle of *Stichopus*, a structure reported to be an axocoel in other holothurians (Bury, 1895), is a calcite secreting syncytium and not an epithelium, which the definition of a putative coelomic tissue requires (Hyman, 1955). Since the madreporic vesicle is a syncytium in *Stichopus*, it is not homologous with the axocoelic coelom of asteroids, ophiuroids, and echinoids. Therefore, I conclude that there is no separate axocoel in this animal. My interpretation argues that Bury was incorrect in asserting that the madreporic vesicle of *Labidoplax* was the holothurian axocoel.

The madreporic vesicle of *Stichopus californicus* lies around the hydroporic canal, close to the body wall. Since there are no intercellular junctions between the cells of the madreporic vesicle and those of the hydroporic canal, and since calcite secreting syncytia in other echinoderms are formed from mesenchyme (Okazaki and Inoué, 1976; Gibbins *et al.,* 1969), I suggest that the madreporic vesicle is also formed from mesenchyme. Mesenchyme cells in holothurians arise from the vegetal plate, the archenteron and the enterocoelomic vesicle (Selenka, 1876; Semon, 1888; Bury, 1895; Rustad, 1940). If the madreporic vesicle was an epithelium formed from mesenchyme, it might be a coelom, but it still would not be homologous with the axocoel of the asteroids, ophiuroids, and echinoids. The madreporic vesicle in these classes arises from the axocoelic coelom (Bury, 1895; MacBride, 1914; Hyman, 1955), which divides off from the hydrocoel during development (MacBride, 1914; Hyman, 1955), but remains connected to it through metamorphosis (Fig. 16). My observations indicate that the separation of these anterior coeloms by Bury (1895) may have obscured some of the evolutionary relationships in the phylum. Hyman (1955) recognized this problem and referred to the combination of axocoel and hydrocoel as an *axohydrocoel* to underline their connection.

The axial sinus of asteroids, ophiuroids, echinoids, and crinoids is derived from the coelom (Bury, 1895; MacBride, 1914; Hyman, 1955). In asteroids, ophiuroids, and echinoids, the axial sinus is required for the establishment of the genital rachis, the progenitor of the gonad (Hyman, 1955), but, no genital rachis forms in holothurians (Hyman, 1955; Smiley and Cloney, 1985). The axial sinus is a prominent coelomic component of the axial complex, which also contains a connective tissue component, the axial gland, (Hyman, 1955; Erber, 1983a, b). Erber (1983b) has shown that an axial gland is present near the dorsal hemal structures in several adult holothurians. I have found no evidence of the axial gland in metamorphosing *Stichopus*, indicating that its development must occur after metamorphosis is complete. I have found no evidence for even a transient existence of an axial sinus in *Stichopus*. Since the axial gland forms in crinoids in the absence of an association with the axial sinus, it is reasonable to assume that the condition in *Stichopus* is similar. Erber (1983a, b) however, concludes that a vestigial axial sinus is represented by the madreporic vesicle. The evidence from *Stichopus* does not support this hypothesis. It may be that the perivisceral coelom encloses the madreporite of adult holothurians, or the peritoneum surrounding the madreporite may be a secondary coelomic derivative like the peripharyngeal or perianal coeloms (Hyman, 1955).

With these data, I examine the question of whether the undivided axohydrocoel in holothurians is primitive or derived. Although we cannot directly test these hypotheses, we can make predictions of structure and function based upon them. First, if the undivided axohydrocoel is primitive, we can predict that holothurians would lack structures such as a pentaradial gonad or a transitory axial sinus at metamorphosis; structures that are the direct result of an inductive influence by the separate axocoel in the asteroids, ophiuroids, and echinoids. Further, we can predict that those subsidiary functions subsumed by the axocoel in asteroids, ophiuroids, and echinoids, such as formation of the distal portion of the stone canal and attachment of the larva at metamorphosis, would be subsumed by the axohydrocoel alone in holothurians. But, if the undivided holothurian axohydrocoel is derived, we can predict vestigial structures whose presence reflects a separate axocoel at some time in the past; structures such as pentaradial gonads, or a transitory axial sinus. In addition, the subsidiary functions of the separate axocoel would be subsumed by various organs other than the axohydrocoel alone during the early development or metamorphosis of holothurians. My analysis of the anatomy of *Stichopus californicus* bears out the predictions implicit in the hypothesis that the undivided axohydrocoel is primitive. Of course, it can be argued that the axocoel might have divided off from the axohydrocoel and either been lost or regressed leaving no trace of its existence, but this hypothesis offers no explicit predictions, and cannot be tested.

The importance of the holothurian buccal podia to these considerations is underlined by the general agreement that an ancestral function of the echinoderm water vascular system was to support circumoral feeding tentacles (Bather, 1900; Hyman, 1955; Beklemischev, 1969; Paul and Smith, 1984). Among extant echinoderms, holothurians alone retain this original function. The anterior side of the axohydrocoel gives rise to the coelomic lining of the buccal podia during *Stichopus* development. The buccal podia of holothurians have no direct counterpart among the rest of the echinoderm classes (MacBride, 1914). While the buccal podia are primary podia, and they may bear a photoreceptor (Yamamoto and Yoshida, 1978), they are not at the posterior end of a radial canal as are the primary azygous podia of the remaining classes. These facts led Semon (1888) to hypothesize that the buccal podia of holothurians were homologous with the radial canals of the other classes. This contention caused substantial controversy (Bury, 1895; MacBride, 1914), because it implied that

the radial canals of holothurians were not homologous with the radial canals of other echinoderms. However, MacBride (1914) noted that difficulties remained with the homology of the holothurian buccal podia.

I suggest that the separate axocoel in echinoderms could have arisen from the coelomic lining of buccal podia in an ancestral echinoderm with a coelomic configuration resembling modern holothurians. That is, from the part of the undivided axohydrocoel that was specialized for larval attachment. I make this suggestion because the coelomic lining of the buccal podia is derived from the undivided axohydrocoel, because holothurians lack a separate axocoel, because settling juvenile holothurians attach by their buccal podia, and because the buccal podia of holothurians have no obvious homologous structure in the other echinoderm classes. The transition of the coelomic lining of buccal podia from axohydrocoelic attachment organs (which later develop into adult feeding organs), to axocoelic attachment organs (which are lost in the adult), explains both the function and the remarkable structural similarities of the brachiolar attachment organs with tube feet (Barker, 1978), while retaining the homologous tissues of origin.

Conclusions

This description and analysis of metamorphosis in *Stichopus californicus* demonstrates that there are substantial differences between the traditional view of holothurian metamorphosis (Bury, 1895; MacBride, 1914), and the events described here. These differences include characters that appear primitive when compared to the condition in asteroids, ophiuroids, and echinoids, and that may be ancestral among all echinoderms, such as the relative simplicity of the morphogenic movements associated with metamorphosis, the retention of the larval axis of bilaterality and the absence of metamorphic torsions, the absence of an adult rudiment, the presence of coelomic pores, the presence of an internal madreporite, and the lack of a separate axocoel. These differences are not consistent with the generally accepted notion that holothurians are closely related to echinoids.

The idea of shared globose form or shared meridional growth gradients in echinoids and holothurians, an important argument used in support of the assignment of holothurians to the subphylum Echinozoa, reflects only a superficial similarity between these animals. The most significant contradiction to this concept is the absence of axial or visceral torsion in holothurians. This fact explains the radically different orientation of holothurians compared to echinoids. Holothurians and echinoids are just not comparable globose creatures. It should be possible to derive the globose echinoids from the stellate asteroids and ophiuroids, while maintaining the internal organs in their appropriate position (Smith, 1984b). The distinction between a meridional growth gradient in the echinozoa and a radial growth gradient in the stellarozoa may be based on an incorrect comparison of the apical zone of the echinoid aboral surface and the entire aboral surface of asteroids (Fell, 1963). The larger part of the aboral surface of asteroids can be interpreted as interambulacral rather than apical. As to the question of the proposed homology of the echinoid Aristotle's lantern and the holothurian aquapharyngeal bulb, a definitive answer to this conundrum will require detailed functional morphological analysis.

In summary, each point in this study contradicts the hypothesis that holothurians and echinoids shared a common ancestor. The assignment of holothurians to the subphylum Echinozoa should be reassessed. Since this paper argues that holothurians are primitive only in comparison with asteroids, ophiuroids, and echinoids, since this is based only on data derived from metamorphosis, and since Smith (1984a) has

reach... contrary conclusions, it is prudent to further test the hypothesis that holothurians are primitive echinoderms by constructing a suite of characters, including those described here, and subjecting it to computer assessment for the most parsimonious phylogenetic tree (Smiley, 1986).

ACKNOWLEDGMENTS

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